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1105/29

From: Brumback, Brenda
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Please send:

Sequence of RNA2 of the *Helicoverpa armigera* stunt virus (Tetraviridae) and bacterial expression of its genes.
Hanzlik T N ; Dorrian S J; Johnson K N; Brooks E M; Gordon K H
CSIRO Division of Entomology, Canberra, Australia. terryh@ento.csiro.au Journal of general virology (ENGLAND) Apr 1995, 76 (Pt 4) p799-811, ISSN 0022-1317 Journal Code: 0077340

Molecular analysis of the *Heliothis armigera* stunt virus (Tetraviridae)
Hanzlik, T.N.; Bawden, A.; Dorrian, S.; Johnson, K.; Gordon, K. CSIRO, Australia
12th Annual Meeting of the American Society for Virology 9335024 Davis, CA (USA) 10-14 Jul 1993
American Society for Virology, Abstracts Paper No. 21-10

Thank you,

Brenda Brumback
Brenda Brumback
Art Unit 1642
CM1 Office: 9D03; Mail 8E12
306-3220

C4V
S/30

ABSTRACTS

21-7

STRUCTURAL ORGANIZATION OF A 17 KB FRAGMENT OF *SPODOPTERA EXIGUA* NUCLEAR POLYHEDROSIS VIRUS

E.A. van Strien, H. Gerrits, P. Kucsar¹, A.M. Feldmann², D. Zuidema and J.M. Vlak. Insect Virus Section, Department of Virology, Agricultural University and the Research Institute for Plant Protection¹, Wageningen, THE NETHERLANDS, and the Institute for Plant Protection¹, Budapest, HUNGARY.

The nuclear polyhedrosis virus of *Spodoptera exigua* (SeMNPV) is a highly virulent baculovirus and specific for beet armyworm. SeMNPV is an effective agent to control this pest insect.

The DNA of this virus has been cloned and a physical map has been constructed. A 17 kb fragment containing among others the polyhedrin and p10 genes has been sequenced. The polyhedrin gene (738 nt) is closely related to that of *S. frugiperda* and has been characterized in detail (E.A. van Strien *et al.*, J. Gen. Virol 1992, 73: 2813-2821). An upstream ORF603 homologue of *Autographa californica* (Ac) MNPV has not been found. The p10 gene (264 nt) has no sequence homology with other baculovirus p10 genes, but the size and secondary structure appear to be conserved (Zuidema *et al.*, J. Gen. Virol. 74: 000). Upstream of p10, an ORF (780 nt) equivalent in size but with low sequence homology to the AcMNPV p26 gene was found, whereas downstream an ORF (1956 nt) homologous to the AcMNPV p74 gene was observed. The p26-p10-p74 gene arrangement therefore appears to be highly conserved.

The area between the polyhedrin and p10 genes in SeMNPV is much smaller (11 kb) than in AcMNPV (19 kb). At least two major ORFs were found. One is the homologue of the AcMNPV IE-1 gene; the other ORF has a hitherto unknown function. Homologues to the AcMNPV PE-38, IE-N and ME-53 genes have not been detected. Transcriptional analyses of this area are being carried out.

21-9

The activation of an entomopoxvirus late promoter by the *Autographa californica* nuclear polyhedrosis virus.

Miller, D.P., Palmer, C.P., Marlow, S.A., King, L.A.

School of Biological and Molecular Sciences, Oxford Brookes University, Gipsy Lane, Oxford, OX3 0BP, UK.

A transfer vector was constructed containing the upstream promoter (1 kbp by PCR) and downstream (1 kbp by PCR) sequence of the *Amata moorei* entomopoxvirus spheroidin gene, based on the published data of Hall, R. & Moyer, R. (J. Virol 65 p6516 1991). A cloning site (*Bam*H I) was introduced downstream of the spheroidin promoter to facilitate the insertion of coding sequences. In this study we inserted the easily assayable reporter gene chloramphenicol acetyl transferase (CAT). Transient expression of the CAT gene was demonstrated in AmEPV-infected *Estigmene acrea* cells and also in *Autographa californica* nuclear polyhedrosis virus-infected SF21 cells. Transient expression was not observed in non-infected *E. acrea* or SF21 cells. This suggests that transcription factors present in AcNPV-infected cells are able to activate an entomopoxvirus late promoter. These transient observations have been confirmed by the production of a recombinant AcNPV in which the wild-type polyhedrin gene promoter has been replaced by that of the AmEPV spheroidin gene. The spheroidin gene promoter within the AcNPV genome was able to drive the expression of CAT in SF21 cells.

21-8

Identification of late expression factors located between 56.0 and 64.0 map units of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) genome

A. Lorena Passarelli¹ and Lois K. Miller^{1,2}

Department of Genetics¹ and Department of Entomology²
The University of Georgia, Athens, GA 30602

We have previously reported the identification of several AcMNPV genes involved in late and very late transient gene expression (late expression factor or *lef* genes) using an overlapping library of clones that spans all of the AcMNPV genome and a reporter gene driven by an AcMNPV late or very late promoter. We now report the identification of three additional *lef*'s located between 56.0 and 64.0 map units of the AcMNPV physical map. One of these genes was the helicase homologous gene which has been previously characterized and shown to be required for viral DNA replication. The second gene, named *lef-5*, located immediately downstream of the core protein gene has been previously identified by DNA sequencing (open reading frame 6, Lu & Carstens 1991, Virology 181: 336-347). Finally, the third gene is in this region required for late gene expression, *lef-4*, was located immediately upstream of the major capsid protein gene, *vp39*. The nucleotide sequence of this region predicted a polypeptide of 464 amino acids with a molecular mass of 54 kDa. Homologs of this gene are also located immediately upstream of the major capsid protein genes of *Orygia pseudosimilans* NPV and *Lymantria dispar* NPV.

21-10

MOLECULAR ANALYSIS OF THE *Heliothis armigera* STUNT VIRUS (TETRAVIRIDAE).

T.N. Hanzlik, A. Bawden, S. Dorrian, K. Johnson and K. Gordon
CSIRO Division of Entomology, Box 1700, Canberra, ACT 2601

A novel insect small RNA virus of the Tetraviridae, the *Heliothis armigera* Stunt Virus (HaSV), was characterized and its genome completely sequenced. Certain features indicate HaSV has a close relationship to plant RNA viruses. It possesses a bipartite ssRNA genome encased in a 36 nm capsid composed of two (63 kDa and 7 kDa) proteins. The two strands of the genome have lengths of 5.5 kb and 2.5 kb and each have a long open reading frame (ORF) that uses >80% of the coding capacity with smaller, overlapping ORFs being present. Predicted secondary structures included distinct tRNA-like and ICR-like formations on each strand, the first such structures discerned in an animal virus. The sequence of the ORF of the larger strand predicts a RNA replicase while two out of frame ORFs on the smaller strand predict a 17 kDa PEST protein preceding a precursor to the two capsid proteins. Despite a 66% AA sequence identity to capsid proteins of another tetravirus, no common antigens were found by antibodies raised to particles of both viruses. Epitope mapping showed the dissimilar middle regions (33% identity) to possess all the antigenicity. The precursor and its two cleavage products were expressed in bacterial, baculoviral and yeast expression systems. Analysis of the expression products showed "capsid" formation by both the precursor and large 64 kDa cleavage product with no processing of the precursor. As the virus replicates with devastating effect in midguts of infected larvae after oral ingestion, studies of the effect of gut juices and proteases on the stability of capsids and capsoids were conducted.